PIEZOELECTRIC AND RELATED PROPERTIES OF HYDRATED COLLAGEN

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ABSTRACT Two piezoelectric constants (polarization per unit stress, $d = d' - id''$, and polarization per unit strain, $e = e' - ie''$), the elastic constant, and dielectric constant are determined for oriented collagen at different hydration levels at 10 Hz from −150 to 50°C. With no hydration (~0% H_2O), d' increases slightly with higher temperatures, while e' decreases slightly. Near 11 wt% H_2O, both d' and e' increase then decrease around 0°C, and is probably caused by an increase of the dielectric constant and the ionic conductivity in the nonpiezoelectric phase. Hydration greater than 25 wt%, d' and e' decrease above −50°C which is considered to be due to a greater ionic conductivity surrounding the piezoelectric phase.

INTRODUCTION

The piezoelectric and pyroelectric properties of collagen has been investigated in complex biological systems such as bone and tendon (1–6). Relaxational properties of piezoelectricity has been observed in many biological and synthetic polymers (7, 8). But to obtain molecular interpretations, studies on purified collagen are required. Dielectric relaxations (9–12) and mechanical properties of collagen (13–16) have been studied. It is known that piezoelectric constants of collagen vary significantly with temperature and hydration (6, 17). It was decided to investigate oriented collagen sheets and determine their piezoelectric, elastic, and dielectric constants at selected hydration and temperature levels.

EXPERIMENTAL

Uncrosslinked collagen tapes (2 mm wide and 0.09 mm thick) were prepared by extrusion of a dispersion of purified swollen collagen fibrils. During manufacture of the tapes the collagen fibrils are oriented almost exclusively longitudinally. Lengths of the tapes were joined together to increase their width using water as an adhesive. Experimental sheets were constructed with the dimensions of 15 × 15 × 0.16 mm. Thereupon a rectangular sheet was cut 45° to the orientation of the collagen fibrils in order to apply a shear strain. The final dimensions of this sheet was 10 × 5 × 0.16 mm. Finally, silver (5 × 5 mm, area) was evaporated at the center on both surfaces to serve as electrodes.

Measurements of elastic constant, dielectric constant, and piezoelectric constants were conducted at 10 Hz with a temperature range of −150 to 50°C. A schematic diagram of the measurement apparatus (7) is shown in Fig. 1. The sample is vibrated by a driver activated by AC current through the coil. Oscillational force (F) and displacement (Δl) are detected by a piezoelectric ceramic element and a nonbonded type strain gauge, respectively. Oscillational charge (Q) occurring in the sample is then amplified to a voltage (V_Q) by means of a charge amplifier with low input impedance. However, when an amplifier with high input impedance is employed, the AC voltage of the sample (between electrodes) is detected. The operational circuit (Fig. 1) determines the ratio of two input signals, V_x and V_y, as a complex quantity $V_x/V_y = V_z' + iV_z''$.

The real and imaginary components of the ratio of polarization (V_Q) to stress (V_F) give real and imaginary components of the complex piezoelectric strain-constant, $d = d' - id''$. The ratio of polarization (V_Q) to strain ($V_{\Delta l}$) determines the complex piezoelectric stress-constant, $e = e' - ie''$. The complex elastic constant, $c = c' + ic''$, is obtained from stress (V_F) to strain ($V_{\Delta l}$) ratio. When a high input impedance amplifier is used instead of the charge amplifier, the signal (V_v), which is proportional to the voltage of the sample between the electrodes is obtained. In the present experiments d and e constants are determined. However, other piezoelectric constants, ratio of piezoelectric field to stress, $g = g' - ig''$, and ratio of piezoelectric field to strain, $h = h' - ih''$ could be determined.

The complex dielectric constant, $\epsilon = \epsilon' - i\epsilon''$, was measured by the circuit illustrated in Fig. 2. An AC voltage at 10 Hz is applied to the collagen sheet. The AC current through this sample is integrated by a charge amplifier, and its output goes to two phase-sensitive detectors (PSD). The reference signals of the PSD have phases 0 and $\pi/2$ relative to the oscillator voltage. Thus the PSD gives ϵ' and ϵ'', which may be determined at different temperatures.

Collagen samples were placed in desiccators containing at chosen relative humidities

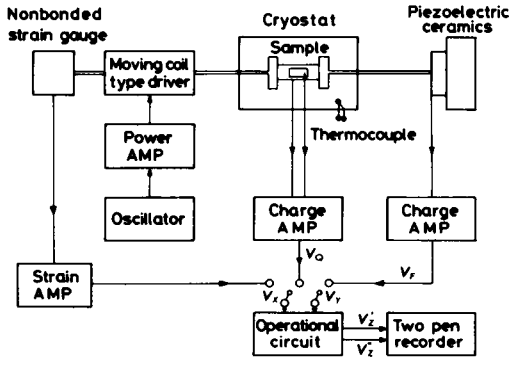

FIGURE 1

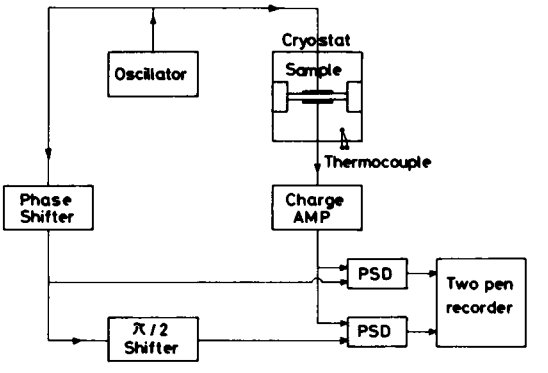

FIGURE 2

FIGURE 1 A schematic diagram of an apparatus measuring piezoelectric constants.
FIGURE 2 A schematic diagram of an apparatus measuring the dielectric constant.

(RH) of 6.5, 23, 43, 63, 75, 84, and 93%. These samples were kept at a humidity for at least 2 days before measurements.

Sample no. 1 (RH = ~0%) being more specialized was treated as follows: The sample was affixed to clamps inside a measuring cell, at the bottom of which silica gel was placed. Dried nitrogen gas saturated this cell. Then the sample was heated to about 120°C for about 3 h and subsequently cooled to about −150°C by liquid nitrogen. Owing to these drying conditions, sample no. 1 probably contains a trace of absorbed water.

RESULTS

The temperature dependence of d constant and e constant at 10 Hz are shown in Fig. 3 and Fig. 4, respectively, for different hydrated collagen sheets. If rectangular coordinates are assigned to samples, so that the 3-axis is in the direction of collagen fibrillar orientation and the 1-axis is normal to the surface of the collagen sheet, the piezoelectric constant, d_{14}, is determined. Note d_{14} is negative which accords to pre-

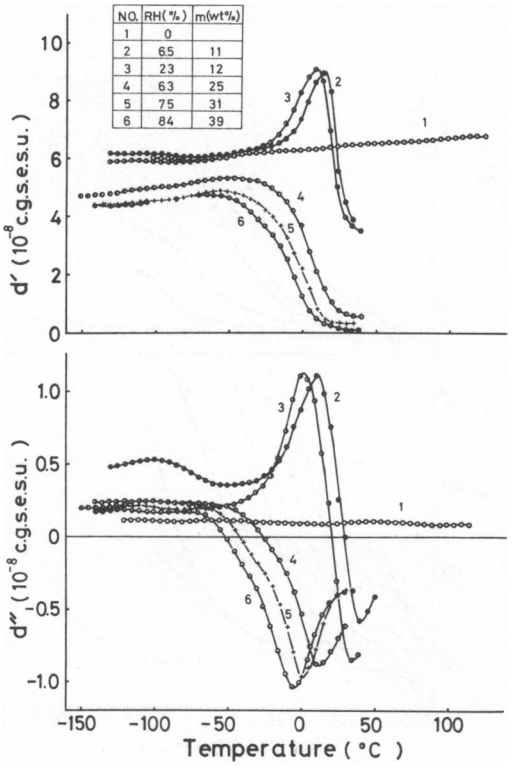

FIGURE 3

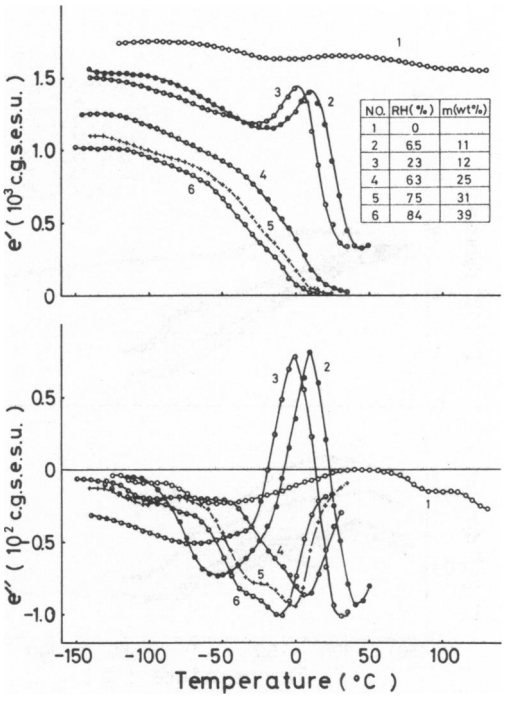

FIGURE 4

FIGURE 3 Temperature dependence of the piezoelectric strain-constant, $d = d' - id''$, for oriented collagen sheets with different hydration.

FIGURE 4 Temperature dependence of the piezoelectric stress-constant, $e = e' - ie''$, for oriented collagen sheets with different hydration.

vious results (4, 5). The piezoelectric constant, $d = d' - id''$, of this study gives the ratio of polarization to stress at 45° to the direction of fibrillar orientation in the collagen and equal to $-d_{14}/2$.

In sample no. 1, d' increases slightly and e' decreases slightly with increasing temperature. In samples no. 2 and no. 3, both d' and e' show a maximum near 0°C. For samples nos. 4–6, d' increases and then at −50°C begins to gradually decreases with increasing temperature. Corresponding to this change of d', d'' is positive (delayed phase) below −50°C and exhibits a negative maximum (leading phase) around 0°C. While e' gradually decreases with increasing temperature, e'' is negative and shows a negative maximum near 0°C. Notice when temperature increases, an increase of d' or e' is accompanied by positive d'' or e'' (lagging phase); whereas a decrease of d' or e' is accompanied by a negative d'' or e'' (leading phase). This suggests that the principle of temperature-time equivalence is valid for piezoelectric relaxations.

Temperature dependence of the complex elastic constant, c' and c'' at 45 degrees to the direction of the collagen fibrillar orientation is shown in Fig. 5. The dynamic elastic constant c' decreases with increasing hydration. The temperature dependence of

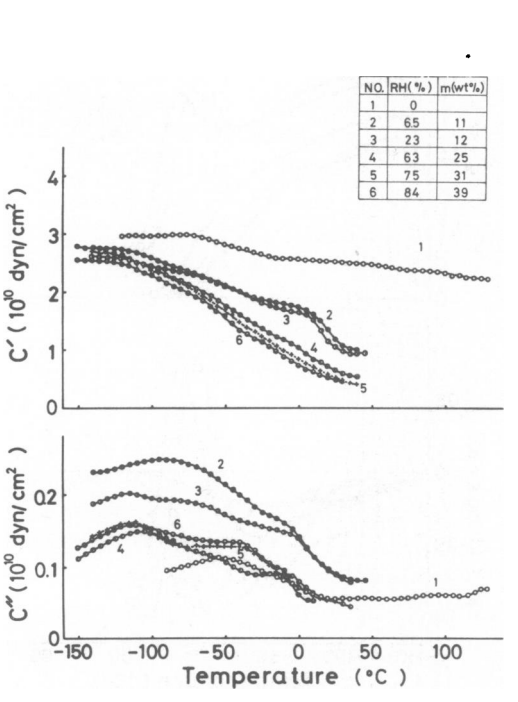

FIGURE 5

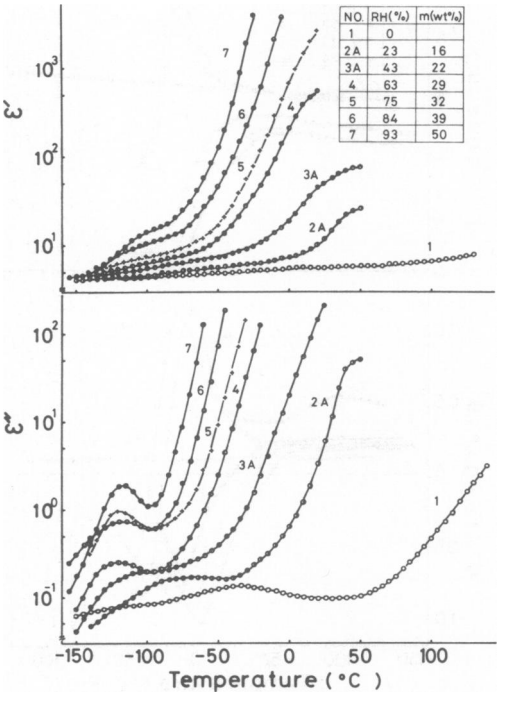

FIGURE 6

FIGURE 5 Temperature dependence of the elastic constant, $c = c' + ic''$, for oriented collagen sheets with different hydration.

FIGURE 6 Temperature dependence of the dielectric constant, $\epsilon = \epsilon' - i\epsilon''$, for oriented collagen sheets with different hydration.

the complex dielectric constant, ϵ' and ϵ'' is shown in Fig. 6. Note, ϵ' and ϵ'' increase sharply with increasing hydration.

DISCUSSION

Lüscher et al. (18) investigated collagen hydration with X-ray diffraction and reported that the adsorption of water by the helical structure of the collagen molecule becomes saturated at a moisture content of 26 wt% (RH = 60%). Above this moisture content water is adsorbed within the intermolecular space (19). Infrared absorption intensity at 1,550 cm^{-1} (amide II) increases with an increase in moisture content until about 25 wt% and then levels off (20). Observe in Figs. 3, 4, and 5 that the data of *d*, *e*, and *c* for the samples with moisture contents higher than 25 wt% exhibit a different temperature dependence than those with lower moisture contents.

Hydration's influence on molecular motion of collagen has been investigated by measuring dielectric properties (11, 12), elastic properties (14–16), and nuclear magnetic resonance properties (16, 21). Chang and Chien (12) reported dielectric relaxations at −80°C, 10°C, and 88°C at 100 Hz for reconstituted collagen with 12 wt% moisture content. They observed elastic relaxations at −80°C and 18°C at 110 Hz (15). Baer et al. (14) observed elastic relaxations around −90°C, −10°C, and 130°C at about 1 Hz for collagen with 10 wt% moisture content. Stefanou et al. (16) reported elastic relaxations at −80°C and −20°C at 1 Hz for collagen with 10 wt% moisture content. They also noted two step decreases of second moment in nuclear magnetic resonance at −70°C and 0°C for collagen with 6.5 wt% moisture content. One may conclude from these experimental results that for collagen with 10 wt% moisture there exist two kinds of relaxations in dielectric and elastic properties at −80°C and 0°C. These relaxation temperature positions vary due to differences in the sample, moisture content, and measuring frequency. Thus interpretations for the origins of these relaxations remain inconclusive. However, a plausible explanation may be that the relaxations at 0°C result from reorientational motion of the collagen molecule's side chains, whereas relaxation around −80°C is caused by low frequency oscillation of the collagen's main chain, but all correlated to the adsorbed water (16).

As seen in Fig. 5, sample no. 2 (11 wt% of moisture content) shows a maxima of c'' at −90°C and −10°C. Temperature maxima shift toward a lower temperature with an increase of moisture. We assume that the dispersion at −90°C is associated with the local mode relaxation of main chains but that at −10°C with the side chain relaxation. The local mode relaxation is indicated by maxima of ϵ'' at low temperature (Fig. 6).

But samples no. 2 and no. 3 indicate a noticeable change of *d* and *e* constants (Figs. 3 and 4 at 0°C), while d' and e' initially increase and then decrease with increasing temperature, accompanied by positive and negative maxima of d'' and e'', respectively. These variations of *d* and *e* with temperature are similar to those reported in oriented films of poly-γ-methyl-glutamate (22) and poly-γ-benzyl-glutamate (23) at temperatures coinciding with their side chain relaxation. Similar temperature dependence of *d* and *e* is also observed for films of mixtures of polyvinyl alcohol (PVA) and piezoelectric ceramic powders (PZT) at the glass transition temperature of PVA (24). When the

moisture content is less than 25 wt%, water is adsorbed mainly to side chains and main chains of helical molecules and does not fill the intermolecular space. In general, piezoelectric polymers consist of a piezoelectric, crystalline phase, and a nonpiezoelectric, noncrystalline phase. When an external stress is imposed on a sample, polarization is produced in the crystalline phase inducing a measurable charge on the sample's surface. If the dielectric constant in the noncrystalline phase surrounding the crystallites increases with temperature, the induced charge on the surface of the sample also increases, and thus d' and e' will increase with temperature. However, at higher temperatures ionic conductivity on the surface layer of crystallites increases and neutralizes the polarization of the crystalline phase; thereupon d' and e' decreases with increasing temperature.

When the moisture content is larger than 25 wt%, ϵ' and ϵ'' increase remarkably with increasing temperature as shown in Fig. 6. Water is adsorbed in the intermolecular spaces and functions as a plasticizer. Temperature-time equivalence is valid for samples with high moisture contents. The experimental data of ϵ' and ϵ'' for sample no. 6 with 39 wt% moisture content were plotted against measuring frequency in Fig. 7. Curves were shifted horizontally to make a single composite curve at $-20°C$, which is

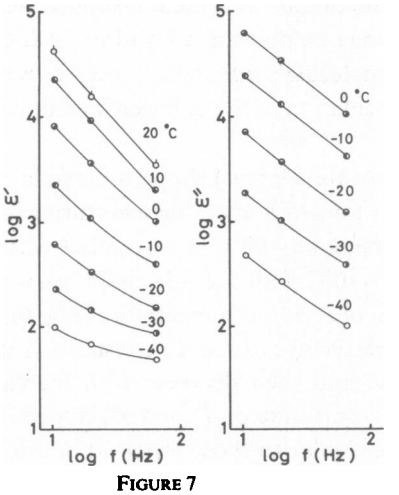

FIGURE 7

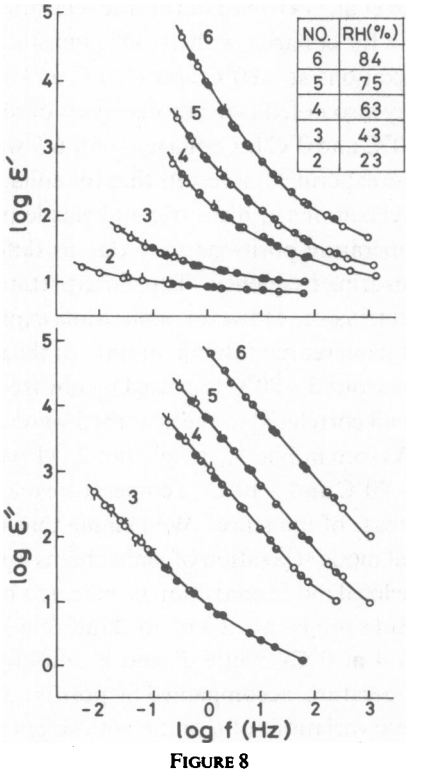

FIGURE 8

FIGURE 7 Frequency dependence at different temperatures of the dielectric constant, $\epsilon = \epsilon' - i\epsilon''$, for an oriented collagen film with 39 wt% moisture content.

FIGURE 8 Master curves of the dielectric constant, $\epsilon = \epsilon' - i\epsilon''$, at $-20°C$ and different moisture contents.

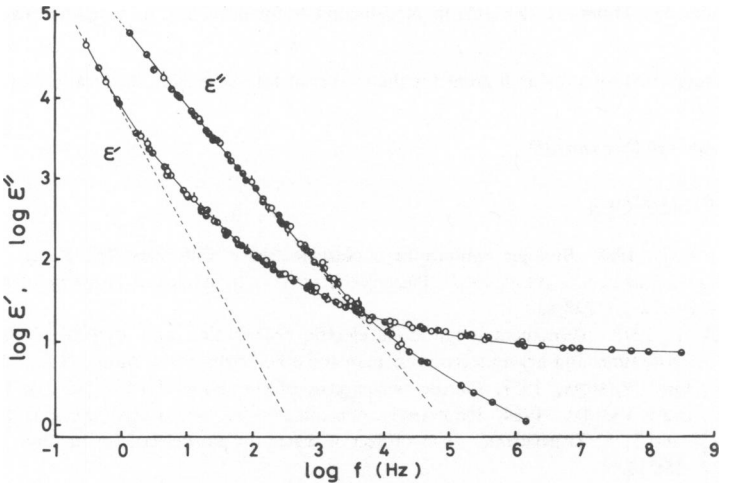

FIGURE 9 Master curves of the dielectric constant, $\epsilon = \epsilon' - i\epsilon''$, at $-20°C$ and 39 wt% moisture content.

shown in Fig. 8. Thus the curves in Fig. 8 are master curves at $-20°C$ derived for different moisture contents. These curves could be also shifted horizontally making a single composite curve (Fig. 9) and represent master curves of ϵ' and ϵ'' at $-20°C$ and 39 wt% moisture content. The larger values of ϵ' and ϵ'' show the influence of ionic conductivity of water. Uemura (25) proposed that ϵ' and ϵ'' are proportional to $f^{-3/2}$ and f^{-1}, respectively, where f is the frequency, when the contribution of ionic conduction to ϵ' and ϵ'' is significant. It is seen in Fig. 9 that at the lower frequency range the above relations hold for master curves of ϵ' and ϵ''. This supports the conjecture that ionic conduction is important in the dielectric and piezoelectric properties of collagen.

The constants, e' and d' for samples nos. 4, 5, and 6 (Figs. 3 and 4) markedly decrease above $-50°C$ with increasing temperature. The piezoelectric polarization is produced within the crystalline phase of the sample which is surrounded by the nonpiezoelectric and noncrystalline phase. When the moisture content becomes large, water is adsorbed on the crystallite's surface and produces a conductive layer. At $-50°C$ side chain relaxation takes place and the mobility of ions increases in the adsorbed water layer on the surface of crystallites. The polarization on the crystallite's surface is neutralized by this surface conductivity. Both d and e constants should thus decrease to zero with increasing temperature. The curve shifts to a higher temperature upon decreasing moisture content. At temperature below $-50°C$, d' gradually increases with increasing temperature (Fig. 3), and d'' is positive, which indicates polarization lags behind the stress. Alternatively, e' gradually decreases with increasing temperature (Fig. 4), and e'' is negative, which indicates polarization precedes stress. These results may be explained by considering an elastic relaxation caused by temperature in the nonpiezoelectric phase surrounding the piezoelectric crystalline phase (22). Since $d = e/c$, if e is unchanged with temperature, the decrease of c should cause an increase of d.

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